# THE IDENTIFICATION OF THE MOST CHARACTERISTIC SALIVARY ORGANISM, AND ITS RELATION TO THE POLLUTION OF AIR[1]

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## INTRODUCTION

Bacteriologists and sanitary engineers have, within the last score of years, given much attention to the detection of excremental pollution in water. They have shown that by making it possible to recognize certain characteristic accompanying organisms, bacteriological methods are capable of revealing this kind of pollution even when it exists to such a small degree as to be beyond the range of chemical detection. Small as these quantities of contaminating substances may seem, they may nevertheless endanger the health of a whole community by exposing it to possible pathogenic organisms derived from the excreta of a diseased host.

It is not merely by the aggregate bacterial yield that the potability of a water in its relationship to disease is judged, but more specifically by the species of bacteria present, and their relative abundance. The micro-organisms which serve as an index of pollution, and for which special quantitative examination is made, are the members of the colon group. These, from their constant presence and relative abundance, are characteristic of material of excremental origin. Their presence in water in sufficient quantity indicates pollution, and their relative abundance serves as an index to the extent of the latter.

Bacteriological technique has not as yet been applied to the same extent in the detection of pollution in air. Chemistry has, up to the present time, been of more practical value here.

[1] An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis in partial fulfillment of the requirements for the degree of master of arts in the Henry Shaw School of Botany of Washington University.

The proportion of carbon dioxide is still the standard mainly relied on for estimating pollution of air by materials given off from the human body, although it is recognized that other factors may be of more importance. This method of examining air, however, is of little or no value in furnishing an index to the probable or possible contamination with disease-producing germs, for there is at present no reason for believing that such organisms are given off in the breath during ordinary quiet breathing. Thus, M. H. Gordon[1] calls attention to the following: Tyndall observed that expired air is optically purer than inspired air; Cornet found air expired by tubercular patients to be free from the tubercle bacillus; and Straus has shown that expired air is not only comparatively free from bacteria, but that it is considerably purer in this respect that inspired air. It nevertheless appears probable that bacteriology rather than chemistry will furnish a means of investigating the pollution of air by disease-producing germs. The problem at hand is to devise, if possible, a method for estimating the degree of pollution of air by pathogenic organisms (given off from the human body) in a manner similar to that employed in estimating the extent of pollution of water by similar organisms of excremental origin.

## HISTORICAL

It appears that the present status of bacteriological analysis of air is comparable to that of bacteriological analysis of water some years ago, when the total number of bacteria in a given quantity was the chief factor determined. There are various ways in which pathogenic organisms may gain access to the air and ultimately to another individual. In addition to transfer by direct contact, disease-producing organisms may be given off in the urine, in feces, in sputum, or from the surface of the skin. Recently, also, attention has been called to the possibility of the pollution of air by the scattering of fine particles of mucus and saliva from the mouth in the acts of coughing, sneezing, and loud speaking. The latter methods of air pollu-

[1] Report on a bacterial test for estimating pollution of air. Supplement to the Thirty-second Annual Report of the Local Government Board (London), containing the Report of the Medical Officer for 1902–3. 421–471. 1904.

tion are the ones to be considered in this investigation. They doubtlessly constitute an important means whereby pathogenic organisms enter the air from an infected person, subsequently to be transmitted to other individuals.

The discharge of sputum furnishes the most obvious way whereby pathogenic organisms may be expelled from the mouth. The expectorated mucus dries, and, in the form of dust, may later be inhaled to produce infection. The work of Flügge and members of his school,[1] however, has drawn attention to a more direct and no less important way by which germs may be aërially conveyed from the mouth. The problem of transmission of micro-organisms by means of particles of mucus expelled from the mouth in various expiratory acts, was attacked in two ways by the investigators referred to above: 1. The mouth was artificially infected with a culture of *Bacillus prodigiosus*. This organism was chosen because the red pigmentation of the colonies renders the identification easy. After agar plates had been placed at various distances from the person being experimented upon, the individual proceeded to speak, cough, sneeze, etc. At the end of the experiment the agar plates were covered and incubated at 25° C. for 3 days, during which time the characteristic red colonies of *B. prodigiosus* made their appearance. The possibility of error due to the previous presence of this organism in the air of the room was excluded by exposing a series of agar plates immediately before the experiment began, with the result that in all cases the organism failed to appear. The length of time that droplets of mucus remained suspended in the air after the several expiratory acts was determined by exposing plates at various periods after the experiment had been completed. 2. Glass slides or empty Petri dishes were placed at various distances from a tubercular patient. The droplets of mucus expelled during coughing, and deposited upon the glass slides, etc., were either examined microscopically or were washed off and injected intraperitoneally into guinea-pigs. In the former case a bacillus giving the characteristic staining reaction of the tubercle bacillus was found, and in the latter the development of tuber-

[1] Gordon, *loc. cit.*

culosis in the inoculated animals resulted. In other experiments, guinea-pigs, instead of being inoculated, were directly exposed to the coughing of tubercular patients with the result that a number of the animals so exposed contracted tuberculosis. Varied and repeated experiments along these lines established the fact that in the acts of coughing, sneezing, and loud speaking, fine droplets of mucus are ejected into the air, that they float about and may be wafted by air currents, such as obtain in ordinary rooms, to a distance of from 24 to 40 feet.

The most thorough investigation in recent years of the problem of air pollution with micro-organisms was made by Dr. M. H. Gordon.[1] This author believed that the positive recognition of disseminated saliva constituted an important step in the development of an applicable bacteriological method for the examination of air. By bacterial analyses of a number of samples of saliva obtained from normal individuals, Dr. Gordon determined that the streptococci are the organisms most abundantly present in saliva. Of these he was able to differentiate four morphologically different types—*longus*, *medius*, *brevis*, and *conglomeratus*. In endeavoring to differentiate these organisms on a physiological basis a study was made of their virulence, relation to oxygen, optimum growth temperature, pigment production, motility, gelatin liquefying power, indol production, action on litmus milk at 37°C., and action on various carbohydrates.

It was found that the micro-organism which is most useful in the detection of droplets of saliva is *Streptococcus brevis* because it is the only one among the salivary cocci found which changes the color of neutral red broth to yellowish green, and produces acid and clot in milk. Having developed a means of differentiating the coccus most characteristic of saliva, Gordon next examined the open air for the presence of micro-organisms characteristic of saliva. In these experiments broth plates were exposed for a stated length of time and incubated anaërobically at 37°C. In but very few cases were the organisms isolated from the air.

A further means of differentiating the characteristic salivary

[1] *Loc. cit.*

coccus from the air cocci was sought in the action of the two on various organic substances. In this capacity the several broths containing lactose, syringin, and coniferin, proved especially serviceable. In lactose broth the typical salivary coccus was positive, i. e., it produced acid, whereas the air cocci were negative. In the syringin and coniferin broths, the air cocci were positive, the typical salivary coccus negative.

To determine whether or not particles of saliva were disseminated through the air during the acts of coughing, sneezing, and loud speaking, Gordon performed experiments in a large and in a small room, using, at first, Flügge's method of artificially infecting the mouth with a living culture of *Bacillus prodigiosus* and placing sterile agar plates at various distances in front of and behind the speaker. After ½–1 hour of loud speaking, it was found that *B. prodigiosus* had been disseminated to a distance of 40 feet in front of and of 12 feet behind the speaker. In other experiments in which no artificial infection of the mouth was resorted to, but in which the characteristic salivary coccus served as the index of dissemination, it was found that after ¼–1 hour of loud speaking *Streptococcus brevis* appeared on broth plates placed as many as 12 feet in front of and behind the speaker. In similar experiments in which speaking was continued for one hour in an ordinary conversational tone, no dissemination of the salivary *Streptococcus* could be detected.

From his experiments Dr. Gordon inferred that there were certain streptococci normally present in saliva which are applicable for the detection of droplets of saliva in air in much the same manner that *Bacillus coli* can be applied for the detection of fecal matter in water.

## The Identification of the Most Characteristic Salivary Organism

With a view of determining the organism most characteristic of saliva, I have undertaken, as a first step, a bacteriological analysis of the saliva of a normal individual. In this examination special attention was paid to the type of organism most abundantly present. Having determined the type, i.e., whether bacillus, coccus, or spirillum, characteristic reactions for it were next sought in order to render its recognition easy. Since

a possible relation of the characteristic salivary organism to the pollution of air was to be investigated, it was necessary to examine the outdoor air free from human contamination for the presence of micro-organisms closely allied to those characteristic of saliva. As particles shed from the skin may be present in the air, it was further necessary to examine those micro-organisms found on the skin which were closely allied to the ones characteristic of saliva.

In examining the saliva for the type of micro-organism most constantly present, i.e., whether bacillus, coccus, or spirillum, the dilution method was used. It is reasonably safe to assume, after repeated trials, that the type of micro-organism which persists longest in continued dilutions is the type most abundant in the material examined. This is true provided the medium on which the organism is grown is approximately equally favorable for the development of all the types present. The dilutions were carried out as follows: A sample of saliva was collected in a sterile test-tube and 1 cc. introduced into a second tube containing 9 cc. of sterile distilled water. The contents of the latter were then thoroughly mixed and 1 cc. of the liquid introduced into a third tube likewise containing 9 cc. of sterile distilled water. This procedure was repeated until 6 dilutions had been effected. Obviously, 1 cc. quantities of each of the 6 successive dilutions contain respectively $\frac{1}{10}$, $\frac{1}{100}$, $\frac{1}{1,000}$, $\frac{1}{10,000}$, $\frac{1}{100,000}$, and $\frac{1}{1,000,000}$ cc. of saliva.

One plate each from dilutions 4, 5, and 6 was made, 1 cc. of the respective dilutions being introduced into 10cc. of nutrient + 1 agar. After thorough mixing, the plates were incubated aërobically for 24 hours at 37°C. The plate made from dilution 5 produced 20 colonies, whereas the one from dilution 6 showed no growth. From each of the 20 colonies a cover-glass preparation stained with gentian violet was made. Microscopic examination revealed the fact that each of the 20 colonies was composed of micro-organisms of the coccus type. Transfers were then made to agar slopes which were incubated at 37°C., for 24 hours. The cultures obtained in this manner were numbered from 1 to 20 and kept at 20°C., as stock cultures.

In examining the open air, sterile agar plates were exposed as indicated in table 1.

TABLE I

DATA ON THE COLLECTION OF AIR COCCI

| Place of exposure. | Time of exposure | Total colonies on plate after 24 hrs. at 37°C. | Coccus colonies on plate after 24 hrs. at 37°C. | Number given to stock culture | Remarks |
|---|---|---|---|---|---|
| Window sill outside of laboratory, 2nd floor. | 15 minutes. | 14 | 7 | 21 to 27 incl. | |
| On shelf, center of laboratory room. | do. | 2 | 0 | | One person in room. Abundance of *Monilia* present. |
| On table, in reading room. | do. | 2 | 0 | | do. |
| On table, in plating room of laboratory. | do. | 0 | 0 | | *Monilia* suppressed growth. |
| On table, in basement | do. | 3 | 0 | | |
| On lawn, in garden | do. | 9 | 4 | 28 to 31 incl. | |
| In living room. | do. | 7 | 6 | 32 to 37 incl. | |
| Window sill, 4th floor, downtown section | do. | 18 | 0 | | Much soot on plate. |
| On table, in draughting room. | do. | 1 | 1 | 38 | One person in room. |

After exposure the plates were covered and incubated aërobically for 24 hours at 37°C. Stained preparations of all colonies developing were made and examined under the microscope. The coccus forms were transferred to agar slopes, and after incubation for 24 hours at 37°C., were kept in stock at 20°C. Several of the plates exposed in various parts of the laboratory building were rendered worthless by an abundant growth of *Monilia sitophila.*

The method of examining the skin for organisms closely allied to those characteristic of saliva, was as follows: Test-

tubes, each containing 10 cc. of distilled water and a piece of linen 2 inches square, were sterilized in the autoclav for 15 minutes at 15 pounds pressure. Samples were taken from three parts of the body of a normal individual, namely, the calf of the leg, the thigh, and the chest. This was accomplished by briskly rubbing the portion of the body from which the sample was to be taken with the piece of linen held in sterilized forceps, and later replacing it in the tube of sterilized water. From these dilutions, after being thoroughly shaken, about ½ cc. quantities were plated in 10 cc. of nutrient agar. From each plate 2 coccus colonies were selected from which transfers were made to agar slopes. These, after 24 hours at 37°C., were kept as stock cultures at 20°C.

There were now in stock a total of 44 pure cultures of *Coccaceæ*, 20 from saliva, 18 from the open air, and 6 from the skin.

## MORPHOLOGICAL CHARACTERS

The form of the individual cell is of little value in differentiating the species of *Coccaceæ*, for under conditions favorable to their growth, all appear as regular spheres. Irregular oval cells occur at times, but the form usually becomes normal after cultivation. Some writers lay considerable stress on the value of cell grouping in the *Coccaceæ* as a means of differentiation. With the utmost care in cultivation and staining, however, this could not be verified in the cultures under observation. All the cultures examined contained cells occurring singly, in pairs, in short chains, and in masses, but in no case did the cells of any specific culture exhibit a distinct tendency to occur in any one form. A stained cover-glass preparation showed various cell groupings in different parts of the same microscopic field.

Cell grouping was studied in the following manner: An oese of sterile +1 bouillon was placed on a sterile cover glass, inoculated with a 24-hour culture of the organism to be examined, and inverted on a Van Tieghem cell containing a few drops of sterile distilled water. After sealing the cover glass on the cell with vaseline, the preparation was incubated for 24 hours at 37°C. At the end of this time the cover glass was removed and the drop of water containing the organism allowed to

evaporate. Then, 3 drops of mercuric chloride solution were applied and after 2 minutes washed off with distilled water. Following this, the preparation was treated with a few drops of 1 per cent acetic acid for 5 minutes, again washed in water, and finally stained for about 15 seconds with a few drops of gentian violet. After washing, and drying in the incubator at 37° C., the vaseline was removed from the cover glass with xylene, and the preparation mounted in balsam and examined under the microscope. The relation to Gram stain was observed on 2 and 4-day agar cultures incubated at 20°C. The preparations were treated with aniline oil-gentian violet for 1½ minutes, with Gram's iodine solution for 1½ minutes, and finally with 95 per cent alcohol for 3 minutes. The reactions are recorded as "− −" (decolorized in both tests), "+ −" (stained in one test and decolorized in the other), and "+ +" (stained in both tests).

## CULTURAL CHARACTERISTICS

All cultural characteristics were observed in streak cultures on agar slants after 14 days' incubation at 20°C., and 37°C. Such differences as developed between the cultures were almost entirely variations in color and vigor of surface growth. Under the latter, 5 types were distinguished as follows:

1. Growth very faint and veil-like, or forming scattered translucent colonies.
2. Growth better, but still meager.
3. Growth good, but not abundant.
4. Growth abundant.
5. Growth very heavy.

In the study of chromogenesis, apparent differences in pigment production, due to unequal vigor of growth or evaporation, were, so far as possible, eliminated. This was accomplished by examining in each case the same amount of material—a loopfull—spread evenly on white drawing paper having a rough surface. After drying at room temperature, the color of the pigment produced was compared with the colors as given by Ridgway[1].

[1] Color standards and nomenclature. 1912. [Published by the author, Washington, D. C.]

This author uses as a basis the solar spectrum with its six fundamental colors and intermediate hues, augmented by a series between violet and red not in the spectrum.

## BIOCHEMICAL REACTIONS

The production of indol was investigated in 5-day peptone broth cultures incubated at 37°C. One cc. of a 10 per cent sulphuric acid solution was thoroughly mixed with the broth culture, and then 1 cc. of a freshly prepared 0.01 per cent solution of sodium nitrite was carefully run in on top of the mixture. The appearance of a pink ring at the juncture of the nitrite solution with the acid-peptone solution, was regarded as an indication of the presence of indol. A blank determination for purposes of comparison was made in each case. The action on neutral red broth as regards change in color was observed in cultures incubated for 5 days at 37°C., in the presence of hydrogen.

The organisms were further grown in solutions of nitrate broth to determine whether or not reduction takes place, and if so, whether to nitrite or to ammonia. In carrying out the test a tube of nitrate broth was inoculated with the organism to be tested, and incubated for 4 days at 37°C., an uninoculated tube of nitrate broth being similarly treated to serve as a check. At the end of 4 days, 3 cc. of the broth were removed to a clean test-tube, and 2 cc. each of a naphthylamine solution and of a sulphanilic acid solution added. The development of a red color indicates the presence of nitrites, the intensity of the color being proportional to the amount of nitrites present in solution. To test for ammonia in the remaining portion of the culture, a few drops of Nessler's solution were added. The appearance of a yellow color or precipitate indicates the presence of ammonia. In studying the liquefaction of gelatin by the cocci under observation, the extent of the action only was determined. This was accomplished by spreading a suspension of the organism over the surface of gelatin in 10 mm. tubes. It was found that the amount of material used in this inoculation did not affect the total amount of liquefaction, i.e., whether the amount of transferred material was large or

small the extent of the liquefaction after 30 days' growth at 20°C., was the same with any one organism.

In the study of the action on sterile certified milk particular attention was paid to the coagulation of the milk and to the production of acid. Observations were further made on the effect of the organisms on lactose, saccharose, mannite, salicin, inulin, sorbite, raffinose, and rhamnose. The medium in which these organic substances were used was prepared according to Dr. Houston's formula, as follows:

| | |
|---|---|
| Liebig's beef extract | 1.0 per cent |
| Peptone | 1.0 per cent |
| Organic compound to be tested | 1.0 per cent |
| Sodium bicarbonate | 0.1 per cent |
| 10 per cent litmus solution | 1.0 per cent |

The medium, neutral in reaction to litmus, was sterilized for 15 minutes at 15 pounds pressure in 500 cc. containers, from which sterile fermentation tubes, provided with glass caps, were directly filled. In doing this it was necessary to take utmost precautions to obviate any possibility of contamination. The various organic media thus prepared were inoculated, not from an agar slope, but from a 48-hour broth culture. Gas formation and the production of acid in the several media were observed after 3 days' incubation at 37°C.

## DISCUSSION OF RESULTS

A thorough study of the results will now be made with a view of finding, if possible, some characteristic or group of characteristics, morphological or biochemical, which may be used in differentiating the salivary cocci from the coccus forms of the air and the skin.

The cell grouping varies throughout, there being no arrangement characteristic of any particular group. As observed, all the forms occur in groups, chains, and pairs. As regards the deportment of the various organisms toward the Gram stain, it was noted that all of the salivary cocci gave a positive reaction in both tests; of those from the air, 3 were positive in the two tests, 8 alternately negative and positive, and 6 negative throughout; of the skin cocci, 4 were positive and 2 negative

in both tests. This stain, as may readily be seen, is of no differential value here, for, although the salivary cocci react positively throughout, both positive and negative reactions occur among the air and skin forms.

The production of indol among coccus forms is very uncommon. Of the salivary cocci under observation, none produced indol, and of the air and skin forms only one from each group produced it. The change of color in neutral red broth is, apparently, more frequently brought about by the salivary cocci than by the air and skin forms, but this difference is not sufficiently well marked to be of differential value. Of the 20 salivary cocci, 12 produced fluorescence, whereas only 1 of the air and none of the dermal forms produced this change. All of the forms under observation reduced nitrates to ammonia. Of the salivary forms, 14 out of 20; of the air cocci, 5 out of 18; and of the skin cocci, 5 out of 6, reduced nitrates to nitrites. It thus appears that the reduction of nitrates to ammonia is very common among members of the *Coccaceæ*, but that the reduction to nitrites only is variable and not characteristic of any one type.

The average amounts of gelatin liquefied after 30 days' growth at 20°C., are as follows: by the salivary cocci, 2.8 cc.; by the air forms, 1.9 cc.; and by those of the skin, 1.4 cc. Fifteen out of 20 of the salivary organisms, 15 out of 18 of the air forms, and 4 out of 6 of the skin cocci, liquefied gelatin. Summing up the results obtained from the experiments on gelatin liquefaction, it is to be noted that, in general, the salivary cocci liquefy gelatin more readily than do the air or skin forms, but aside from this it is apparent that there is nothing to warrant the use of gelatin as a differential medium.

The results of the experiments on vigor of surface growth on agar slopes at 20°C., and 37°C., are given in table II. While it may be said, in general,—from the results given in this table—that the salivary cocci grow somewhat more vigorously at 37°C. than at 20°C., the air forms better at 20°C. than at 37°C., and the skin organisms about equally well at the two temperatures, the differences are not sufficiently pronounced to impart to the factor of vigor of surface growth any marked value as a differential characteristic.

TABLE II

DATA ON THE VIGOR OF SURFACE GROWTH OF AIR, SALIVARY, AND DERMAL COCCI

| Source of organism | Temperature of incubation | No. of cultures used | Growth characteristics | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | No growth | Very faint | Meager | Good | Abundant | Very heavy |
| Saliva | 20°C. | 20 | — | 3 | 7 | 10 | — | — |
| Air | 20°C. | 18 | 1 | — | — | 4 | 7 | 6 |
| Skin | 20°C. | 6 | — | 1 | 2 | 2 | 1 | — |
| Saliva | 37°C. | 20 | — | — | 7 | 13 | — | — |
| Air | 37°C. | 18 | 2 | 1 | 9 | 6 | — | — |
| Skin | 37°C. | 6 | — | 1 | — | 4 | 1 | — |

In the following enumeration are listed the colors of the various pigments produced by the air, skin, and salivary cocci, the figure on the left having reference to the number in Ridgway corresponding to the particular pigment produced:

Salivary cocci

| | | | |
|---|---|---|---|
| 15″ d | Light pinkish cinnamon | 3 | 15 |
| 15″ c | Intermediate between light pinkish and pinkish cinnamon | 4 | |
| 15″ b | Pinkish cinnamon | 4 | |
| 15″ a | Intermediate between pinkish cinnamon and cinnamon | 3 | |
| 15″ | Cinnamon | 1 | |
| 21′ e | Intermediate between massicot and straw yellow | 1 | |
| 23′ f | Naphthalene yellow | 1 | |
| 19 f | Maize yellow | 1 | |
| 19′ b | Mustard yellow | 1 | |

One gave too little growth for determination of the color.

Air cocci

| | | | | |
|---|---|---|---|---|
| 21′ | f | Massicot yellow | 4 | 16 |
| 21′ | e | Intermediate between massicot and straw yellow | 2 | |
| 21′ | d | Straw yellow | 1 | |
| 21′ | b | Amber yellow | 1 | |
| 19′ | d | Naples yellow | 1 | |
| 19′ | b | Mustard yellow | 4 | |
| 19′ | | Primuline yellow | 1 | |
| 19 | f | Maize yellow | 1 | |
| 19 | d | Buff yellow | 1 | |
| 3′ | b | Light Jasper red | 1 | |

One form did not grow.

Skin cocci

| | | | | |
|---|---|---|---|---|
| 19 | f | Maize yellow | 1 | 5 |
| 19 | d | Buff yellow | 1 | |
| 19 | b | Apricot yellow | 2 | |
| 21′ | e | Intermediate between massicot and straw yellow | 1 | |
| | | White | 1 | |

At first glance the color of the pigments produced by the organisms would seem to furnish one mode of differentiation. In the majority of cases the salivary cocci produced cinnamon colored pigments, whereas pigments of a yellow color were usually produced by the air and skin forms. Closer inspection shows, however, that some of the salivary cocci, as well as the air forms, produce a maize yellow and a mustard yellow pigment; also that a maize yellow pigment and one intermediate between massicot and straw yellow are produced by representatives of both the salivary and skin cocci. It is apparent that these intergradations make the factor of pigment production largely inapplicable as a differential test.

In milk the salivary cocci with one exception produced acid and coagulated the medium, whereas none of the air forms and but one of the skin cocci gave this combined reaction. This attaches to milk considerable value as a differential medium. In the media containing the various organic substances

—sugars, etc., none of the coccus forms produced gas. All but one of the salivary cocci produced acid in the lactose medium, whereas none of the air cocci and but one of the skin forms deported themselves in this manner. This marks lactose broth as another medium of differential value.

The salivary cocci with but one exception produced acid in saccharose, the single exception being the organism which produced no acid in the lactose medium. Two air cocci and one skin form also produced acid in saccharose, but notwithstanding these exceptions, it appears that saccharose is a third valuable differential medium. In the mannite, salicin, inulin, sorbite, raffinose, and rhamnose broths none of the organisms produced acid, thus marking these organic substances as of no value in differentiating the types of cocci under investigation.

## SUMMARY

In reviewing the preceding discussion of results we find three media, namely, lactose and saccharose broths, and milk, which are of value in differentiating the cocci most characteristic of saliva from those of the air and the skin. One of the salivary coccus forms did not produce acid in lactose and saccharose broths and formed neither acid nor clot in milk. This may have been, and probably was, an air or skin form. Among the air cocci are two which vary somewhat from the remaining air and skin forms in that they produce acid in saccharose broth. Neither of them, however, produces acid in lactose broth, nor acid or clot in milk and in these respects they differ markedly from the characteristic salivary forms. Of the skin cocci one gave the characteristic reactions of the salivary organisms, and it is not at all unlikely that this was a salivary coccus. In general, then, it appears that the organism most characteristic of saliva is a coccus form which produces acid in lactose and in saccharose broths, and acid and clot in milk.

## FURTHER TESTS

To further test the validity of the reactions above referred to as furnishing a reliable means of differentiating between salivary cocci and those of other origin, two additional samples of saliva, from two different individuals, were examined,—

one from a middle aged white person (A), the other from a colored person (B).

The samples were collected and treated in a manner similar to that outlined in the early part of this paper. In the first case (A), transfers were made from all colonies on two plates, representing a dilution of one part saliva in ten billion. These subcultures, all of cocci, were numbered from 1 to 17 inclusive.

In the second case (B), transfers were made from 36 colonies which developed on one-third of a plate representing a dilution of one part saliva in ten billion. The entire series of cultures, numbered from 1 to 36 inclusive, although made from 36 colonies from a plate containing a total of 100 colonies, were found to be made up of coccus organisms. After being incubated in + 1 nutrient broth for 2 days at 37°C., each of the cultures from samples (A) and (B) was transferred to the three differential media,—lactose and saccharose broths, and milk. The results recorded in tables III and IV were observed after 3 days' incubation at 37°C. No gas was produced in any of the sugar media. A blank determination gave negative results throughout on the three media.

TABLE III

REACTIONS OF SALIVARY COCCI (A)

| No. of culture | Lactose broth | Sacch. broth | Milk | | No. of culture | Lactose broth | Sacch. broth | Milk | | No. of culture | Lactose broth | Sacch. broth | Milk | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Acid | Clot | | | | Acid | Clot | | | | Acid | Clot |
| 1 | + | + | + | + | 7 | + | + | + | + | 13 | + | + | + | + |
| 2 | + | + | + | + | 8 | + | + | + | + | 14 | + | + | + | + |
| 3 | + | + | + | + | 9 | + | + | + | + | 15 | + | + | + | + |
| 4 | O | O | O | O | 10 | + | + | + | + | 16 | + | + | + | + |
| 5 | + | + | + | + | 11 | + | + | + | + | 17 | + | + | + | + |
| 6 | + | + | + | + | 12 | + | + | + | + | | | | | |

+indicates positive reaction. O indicates negative reaction.

From the above table it is evident that all but one of the coccus forms in series (A) produced acid in lactose and saccharose broths, and acid and consequent clotting in milk. The one exception was probably an air coccus.

TABLE IV

REACTIONS OF SALIVARY COCCI (B)

| No. of culture | Lactose broth | Sacch. broth | Milk | | No. of culture | Lactose broth | Sacch. broth | Milk | | No. of culture | Lactose broth | Sacch. broth | Milk | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Acid | Clot | | | | Acid | Clot | | | | Acid | Clot |
| 1 | + | + | + | + | 13 | + | + | + | + | 25 | + | + | + | + |
| 2 | O | + | O | O | 14 | + | + | + | + | 26 | + | + | + | + |
| 3 | + | + | + | + | 15 | + | + | + | + | 27 | + | + | + | + |
| 4 | + | + | + | + | 16 | + | + | + | + | 28 | + | + | + | + |
| 5 | + | + | + | + | 17 | + | + | + | + | 29 | + | + | + | + |
| 6 | + | + | + | + | 18 | O | + | O | O | 30 | + | + | + | + |
| 7 | + | + | + | + | 19 | + | + | + | + | 31 | + | + | + | + |
| 8 | + | + | + | + | 20 | + | + | + | + | 32 | + | + | + | + |
| 9 | O | + | O | O | 21 | O | + | O | O | 33 | + | + | + | + |
| 10 | + | + | + | + | 22 | + | + | + | + | 34 | + | + | + | + |
| 11 | + | + | + | + | 23 | + | + | + | + | 35 | + | + | + | + |
| 12 | + | + | + | + | 24 | + | + | + | + | 36 | + | + | + | + |

+indicates positive reaction. O indicates negative reaction.

In series (B), 32 out of the 36 cocci reacted positively throughout on the three differential media. The remainder were positive with saccharose, but negative with lactose and milk, agreeing in this respect with the two air cocci to which reference has been made.

The reactions of the organisms from saliva (A) and (B)

further indicate that the production of acid in lactose and saccharose broths, and a similar production, together with clot, in milk, are characteristic reactions of the salivary cocci.

## CONCLUSIONS

From the results of the preceding experiments it appears that a method applicable for the detection of the organisms characteristic of human saliva has been developed.

It must be acknowledged that the number of organisms examined is comparatively small, especially where those of the air and the skin are concerned. An absolute test of the validity of the adopted mode of identification would necessitate the examination of many hundreds of strains of cocci from numerous sources.

Nevertheless, the characteristic reactions of the salivary cocci examined seem to be sufficiently definite to warrant the assumption that the most characteristic salivary organism is a coccus form which produces acid in lactose and saccharose broths, and acid and clot in milk.

## THE RELATION OF THE MOST CHARACTERISTIC SALIVARY ORGANISM TO THE POLLUTION OF AIR

Having identified the most characteristic salivary organism, the next problem is to isolate it from the air. Its frequency of occurrence must also be determined, as this often serves as an index to the degree of pollution. The isolation of the organism and the determination of its frequency of occurrence can be accomplished simultaneously.

Then come the problems (1) of devising an air-collecting apparatus suitable for all occasions, and (2) of determining the quantity of air to be examined and the terms by which the sanitary quality of the air shall be expressed.

In searching for a means of expressing the sanitary quality of air, let us consider the manner in which this is accomplished in drinking water. Authorities differ markedly on this subject. Shall a water be considered safe or unsafe for drinking purposes if *B. coli* is present in a 100 cc. sample, or shall its presence or absence in 10 cc. or 1 cc. samples be taken as the basis for the

classification? In lieu of a definite standard let us assume the following table[1]:

TABLE V

PRESUMPTIVE TEST FOR *B. COLI* IN WATER

| Sanitary quality | cc. 0.01 | cc. 0.1 | cc. 1.0 | cc. 10.0 | cc. 100 |
|---|---|---|---|---|---|
| Safe | O | O | O | O | + |
| Reasonably safe | O | O | O | + | + |
| Questionable | O | O | + | + | + |
| Probably unsafe | O | + | + | + | + |
| Unsafe | + | + | + | + | + |

+ indicates positive presumptive test for *B. coli.*

We shall now endeavor to prepare a similar table for the purity of air, expressed in the number of salivary cocci present in given volumes. In the normal life processes, the volume of air inhaled is obviously much greater than the volume of water consumed, and this fact must be taken into consideration in establishing a criterion for the bacteriological examination of air. It has been estimated that the tidal air, i.e., the air taken in with each inspiration and given out with each expiration, amounts, in a normal adult when at rest, to one-half liter. Assuming the average frequency of respiration to be 15 per minute, the amount of air inhaled in one minute is $7\frac{1}{2}$ liters, in one hour, 450 liters, and in one day, at least 10,000 liters. Taking the average amount of unboiled water drunk in a day as 2 liters, it would appear that 5,000 times as much air as water is required daily. Hence, the following table, based on table v, may be used to express the sanitary quality of air:

[1] Whipple, G. C. On the practical value of presumptive tests for *B. coli* in water. Techn. Quart. 16:18 e. m. 31. 1903.

TABLE VI

TEST FOR CHARACTERISTIC SALIVARY COCCI IN AIR

| Sanitary quality | cc. 50 | cc. 500 | cc. 5,000 | cc. 50,000 | cc. 500,000 |
|---|---|---|---|---|---|
| Safe | 0 | 0 | 0 | 0 | + |
| Reasonably safe | 0 | 0 | 0 | + | + |
| Questionable | 0 | 0 | + | + | + |
| Probably unsafe | 0 | + | + | + | + |
| Unsafe | + | + | + | + | + |

+ indicates positive reaction in the three differential media adopted.

## APPARATUS AND TECHNIQUE

As it was the intention to collect samples of air in places other than the laboratory, a portable apparatus was necessary. As devised, it consists essentially of a sand filter, a support for same with an attachment for alternately opening and closing the exhaust and suction, and a bulb, having a capacity of 16 oz., with the required amount of rubber tubing. (See plate 2.) The sand filter is of the standard type. It consists of a glass tube 100 mm. long and 10 mm. in diameter, fitted with a one-hole rubber stopper, through which passes a piece of 6 mm. glass tubing. This stopper, with its tubing, forms the support for a circular disc of bolting cloth with a 10 mm. layer of very fine clean quartz sand that passes through a 100, and is retained on a 140 mesh sieve.

The support consists of a rectangular piece of wood 12 x 1 x $\frac{3}{4}$ inches, fitted with a double pinch cock arrangement. Clamps for holding the filter in position are also provided. The rubber bulb is connected to the apparatus in such a manner that when pressure is applied to the former and the pinch cock opened, the air contained in the bulb is expelled through the exhaust without disturbing the sand in the filter in any way. This operation occupies but a few seconds of time. Upon releasing the pinch cock, and immediately thereafter the bulb, the air is drawn through the sand.

The volume of air exhausted from the bulb at each pressure was determined as follows: The bulb, filled with water, was weighed. Pressure was then applied, forcing out the water, after which the bulb was again weighed. The difference in weight in grams is approximately the volume of air in cc. exhausted by a similar pressure. In the calibrations the results varied but slightly. By placing the fingers on the bulb in a certain fixed position each time, it was found that the bulb could be made to deliver 300 cc. of air at each exhaustion and, consequently, to receive 300 cc. of air at each release of pressure. It was, of course, necessary to have all joints air-tight, this being accomplished by making all connections with rubber tubing and glass and using plenty of overlap.

The sand filter, after being plugged at both ends with cotton, was sterilized for 30 minutes at 15 pounds pressure. The rubber stopper support was allowed to fit very loosely into the tube during sterilization in order to prevent setting of the rubber. After the apparatus was removed from the autoclav, the stopper was immediately fitted in tightly, thus rendering the connection air-tight. The sand filter was always used within 24 hours after sterilization. It was connected to the support as shown in plate 2.

When operated in public buildings or conveyances, the support, with the filter, was wrapped in stiff paper in such a manner as to permit of the easy operation of the pinch cock and exhaust. The apparatus thus wrapped was held in the left hand and from it heavy rubber tubing passed down the left coat sleeve and then diagonally across to the right coat pocket where it was connected to the bulb. This rendered the whole apparatus inconspicuous. The bulb was operated with the right hand, the pinch cock with the left. A test tube with a sterile cotton plug was always carried, the latter being used to replace the plug which was removed from the intake of the filter at the beginning of the experiment.

The plating was always carried out within 30 minutes after the sample was obtained. The sand from the filter was carefully poured into a 100 cc. flask containing 15 cc. of sterile distilled water. The bolting cloth, which had a tendency to stick to the rubber stopper, was removed with sterile forceps and introduced

into the flask. The contents of the flask were thoroughly shaken and aliquot portions, as shown in table IX, were plated with 10 cc. of +1 nutrient agar. In plating, the introduction of much sand was avoided in the following manner: The end of the pipette was held immediately above the bottom of the flask while the liquid was being drawn up to a point slightly above the graduation mark. After a few seconds, enough of the sandy liquid was allowed to run back into the flask to leave the water just at the mark. During this short interim a large proportion of the sand settled in the tip of the pipette and was returned to the flask as the liquid was lowered to the mark. Blanks were plated several times during the course of the experiments, but no growth developed in any case.

The plates were in all cases incubated for 4 days at 37°C., after which the number of bacterial colonies present in each was determined. Finally, all, or a representative number, of the colonies were examined for the presence of coccus forms. (See table IX.)

The coccus colonies developing on agar are, as a rule, very small and often grow in the deeper strata of the medium. This renders the transfer difficult especially when two are to be made from the same colony—one for the stained preparation and one for the agar slope to be used as a stock culture. The difficulty was partially obviated by subculturing (from all the colonies in certain selected plates) to agar slopes, and incubating the latter at 37°C. After several days an examination served to eliminate the bacilli and moulds, leaving only the coccus cultures which were later examined for the presence of the salivary forms. In this examination the three differential media described above were used.

## SOURCES OF SAMPLES

As the investigation in hand seeks to discover a relation between the presence of a characteristic salivary organism and the pollution of air, it was thought best to collect the samples of air under normal conditions, i.e., conditions which are met with in every-day life.

Public conveyances, on account of their usually crowded condition and frequently inefficient ventilation, suggested themselves

as favorable places for tests. Hence, a local street car was chosen as a source for air samples. The often poorly ventilated but well filled motion picture theatres furnished another supposedly promising sampling place. The third locality chosen as a source for air samples was a local 5 and 10 cent store. It was thought that this would furnish an ideal source of contaminated air because of the large crowds of people who are continually voicing their sentiments and desires. In order to determine whether or not the salivary organism is present in an atmosphere which is not in immediate contact with human beings, and which is open to the ventilation of nature, the fourth sample was taken from the open air.

## DISCUSSION OF THE EXPERIMENTS

The experiments in table IX are arranged according to the dates on which the tests were made. But for convenience in this discussion the experiments will be taken up according to the source of the samples.

*Experiment 1.*—This experiment was carried out primarily to test the apparatus. The air sample was taken in a laboratory on the second floor of an old building. There were usually at least two people present in the room, and practically no ventilation was provided, the doors and windows being constantly closed. The apparatus used differed from that used in the remaining experiments in that two sand filters were used in tandem instead of the usual one. During the 15 minutes of operation, 7,800 cc. of air were drawn through the sand of both filters at the rate of 520 cc. per minute.

The sand of the first filter was introduced into 15 cc., that of the second into 6 cc. of sterile distilled water. Quantities of both solutions were plated with the following results:

TABLE VII

| Filter number | Plate number | Quantity plated | Total no. of colonies | Coccus colonies |
|---|---|---|---|---|
| 1 | 1 | 1 cc. | 2 | 0 |
| 1 | 2 | 1 cc. | 2 | 2 |
| 1 | 3 | 2 cc. | 4 | 2 |
| 2 | 4 | 5 cc. | 0 | 0 |

The reactions of the cocci isolated from the air in the first filter showed that there was one salivary coccus form present. The remaining three gave negative reactions on the three differential media. It should be noted that out of the 15 cc. of solution from the first filter, only 4 cc. were plated. Eight organisms were present in the quantity examined, making a total of 30 in the entire solution. One characteristic salivary coccus form developed in the portion examined, making, according to the law of averages, a total of 4 in the entire solution. The total volume of air examined being 7,800 cc., the frequency of occurrence of the salivary coccus is 1 in 1,950. According to table VI, the sanitary quality of the air of the room was "probably unsafe" at the particular time at which the sample was taken.

EXPERIMENTS 3, 5. 6, 8, 10

These experiments were carried out in a local street car. The same car line was chosen for all of the experiments in order to eliminate as many variables as possible, such as construction of car, capacity, rate of locomotion, etc. The car was of the ordinary "pay-as-you-enter" type now in use in St. Louis. It had a seating capacity of about 44 people, and could accommodate approximately 40 more standing indoors. The air space in the car in question was about 2,500 cubic feet, or approximately 30 cubic feet for each passenger when the car was filled to its capacity.

As the samples were taken at a time when the outside temperature would not permit the windows to be open, the question of ventilation was carefully studied. As is usually the case, the transoms were tightly closed, and only when the front and rear doors of the car were open at the same time was there an opportunity for a complete renewal of the air. This never happens when the car is in motion, and there is probably never a complete renewal of air unless a strong wind is blowing, thus causing a draught when the car is at a standstill, with both doors open. This particular car was provided with four vents in the roof which could be opened or closed at will. In several of the experiments some of the vents were open; in others, all were closed.

The degree of pollution of the atmosphere in such a car de-

pends, of course, on the amount of coughing, sneezing, speaking, etc., of its occupants. A car may be very crowded but if no coughing, etc., is going on, there will, theoretically, be no pollution of the atmosphere from saliva. Again, if there is much talking, etc., among those present, the atmosphere may be greatly polluted by the dissemination of particles of saliva from the mouth.

The samples were always taken in the early morning between the hours of six and seven, when the majority of the laboring class are on their way to work. The tendency of the passengers at this time of the day is to be quiet, as the morning paper is of absorbing interest to a majority. The samples of air were taken in the center of the car, the opening of the apparatus being about 4 feet from the floor level. In these experiments the apparatus described above was used. In all cases 10,800 cc. of air were drawn through the sand filter at the rate of 900 cc. per minute. The sand was introduced into 15 cc. of sterile distilled water and plated as shown in table IX.

The experiments carried out in street cars will now be taken up in order and the results discussed. If it can be shown that the characteristic salivary organism is present in the air of these cars in sufficient quantity, and if it can later be proved that this salivary organism is not present in the open air, it follows that the atmosphere in these cars is being polluted by the dissemination of particles of saliva from the mouth.

*Experiment 3.*—While the air sample was being taken for this experiment, 44 people were seated in the car, but none were standing. Out of the 20 colonies appearing on the plate (see table IX), 9 were of bacilli and 11 of coccus forms. Inoculated into the 3 differential media, 8 of the latter reacted negatively in all three media, 1 negatively on lactose and milk, but positively on saccharose, and 2 gave positive reactions in all three media.

It will be recalled that mention has been made of several organisms, both among the salivary and air cocci, which gave a positive reaction with saccharose, but reacted negatively with lactose and milk. The one above referred to as reacting in this manner is probably one of these unidentified coccus forms which seem to be present in both saliva and air. Out of

the 11 cocci present, therefore, two were of the characteristic salivary type, and as only one-third of the sample was plated, a total of 6 may have been present in the entire volume of air examined, or a frequency of occurrence of 1 in 1,800. According to table VI, the sanitary quality of the air was "probably unsafe."

*Experiment 5.*—During the sampling process for this experiment, 44 persons were seated and approximately 30 standing. Of the 26 colonies which developed on plate 5 (see table IX), 14 were of bacilli, 2 of streptothrix, and 10 of cocci. When transferred to the three differential media, all of the latter gave negative reactions, indicating that the air in the car at the time of this experiment was "safe."

*Experiment 6.*—At the time of sampling, 44 persons were seated and 30 were standing. On account of the large number of colonies present, only a representative sector of plate 1—comprising one-twelfth of the total area—was examined (see table IX). On this area 21 colonies were counted, 5 bacillus and 16 coccus. On the three differential media, 4 of the latter gave negative reactions throughout, 6 were negative on lactose and milk but positive on saccharose, and 6 gave positive reactions on all three media. It follows that 6 salivary cocci were isolated from one-twelfth of the plate, making a total of 72 from the entire plate, or of 1,080 from the total volume of sand solution,—a frequency of occurrence of 1 in 10. According to table VI, the air in the car at the time of the experiment was "unsafe."

*Experiment 8.*—The number of persons seated and standing was the same as in experiment 6. On the plate examined (see table IX), 34 colonies developed—15 bacillus and 19 coccus. On the three differential media the coccus forms reacted as follows: Twelve gave negative reactions throughout, 6 were negative on lactose and milk but positive on saccharose, and 1 was negative on lactose and saccharose but positive on milk. The last form was found, after again staining with gentian violet and examining under the microscope, to be a short bacillus. It is to be noted that 6 organisms of the unidentified coccus type were again present. No characteristic salivary cocci were present, thereby marking the air of this particular car as "safe" at the time of the experiment.

*Experiment 10.*—During this experiment, 44 persons were seated and 15 standing. It was noted that one transom was open. The plate examined (see table IX) gave a total of 12 colonies, of which 5 were of bacilli and 7 of cocci. Of the latter, 6 reacted negatively on all three of the differential media, whereas 1 gave a positive reaction throughout. This makes the frequency of occurrence of the characteristic salivary coccus form 1 in 3,600, and, according to table VI, marks the air in this car as "questionable" at the time of the experiment.

Summarizing the car experiments, it is to be noted that in three out of five cases the characteristic salivary coccus form was isolated, and in such quantity as to mark the air of one "unsafe," that of another "probably unsafe," and of a third "questionable."

EXPERIMENTS 9 AND 11

These experiments were carried out in a local vaudeville house. The construction of the building appeared modern in every respect. The lower floor had a seating capacity of about 2,000, while the balcony accommodated approximately 1,000 people. The house was filled with spectators on the occasions when the samples were taken. Upon inquiry, after the surprisingly good results given below were obtained, it was found that the building was well ventilated by one of the modern appliances for this purpose, whereby the volume of air in the building (about 90,000 cubic feet) was being renewed to a greater or less extent every seven-tenths of a minute. For the collection of the air samples, the same apparatus was used as in the street car experiments, 10,800 cc. of air being drawn through the sand filter at the rate of 900 cc. per minute. The sand was introduced into 15 cc. of sterile distilled water and platings were made as indicated in table IX.

*Experiment 9.*—The air sample was obtained near the center of the lower floor of the building about 60 feet from the stage. The entire lower floor was packed, and in addition about 100 or more persons were standing in the rear. On the plate examined, a total of 14 colonies developed,—3 mold, 9 bacillus and 2 coccus. Molds were very abundant on the other plates. One of the coccus forms reacted negatively on lactose and milk but positively on saccharose, whereas the other gave negative

reactions on all three differential media. The presence of the single unidentified coccus is again noted. No salivary coccus forms were isolated, from which fact it appears that the air in the particular location from which the sample was taken was "safe."

*Experiment 11.*—This sample was taken on the balcony of the building, about 10 feet from the rear wall. Every seat was occupied. As indicated in table IX, two plates were examined. On the first, 7 colonies developed—3 streptothrix, 3 bacillus, and 1 coccus. On the second plate 3 colonies appeared, all of which were of bacilli. The reaction of the coccus was negative on the three differential media, thereby indicating that the sample of air taken was free from salivary coccus forms and therefore "safe."

In summing up the results of the experiments carried out in the vaudeville house it is to be noted that in both cases no salivary coccus forms were found. Table IX further shows that the total number of organisms found per unit volume of air was smaller than in the street car experiments.

### EXPERIMENTS 4 AND 7

These samples were obtained in the basement of a local 5 and 10 cent store. The ceiling was rather low, being only about 9 feet from the floor level, the entire basement having a volume of about 72,000 cubic feet. The samples in these experiments were taken in the midst of a crowd gathered to listen to a singer advertising songs. Little attention was given to the matter of ventilation until after the results of the experiments were obtained. Subsequently, however, investigation revealed the fact that ample provision had been made for ventilation. Transoms at the level of the sidewalk provide openings to the outside; along the inside wall and near the ceiling are revolving fans about 20 feet apart. These keep the air in circulation until it is drawn out by a suction fan situated in one corner, about 2 feet from the ceiling. The same sampling apparatus was used as in the preceding experiments. As before, a total of 10,800 cc. of air was drawn through the sand filter in each sampling at the rate of 900 cc. per minute. The samples were plated as shown in table IX.

*Experiment 4.*—The air sample for this experiment was taken

in the midst of a crowd of about 100 people in front of a counter. On the plate examined, a total of 36 colonies developed—16 bacillus and 20 coccus. Of the latter, 18 gave negative reactions throughout on the three differential media, and 2 reacted negatively on saccharose and milk but positively on lactose, the latter sugar being fermented. No salivary coccus forms were isolated, indicating that the air in the basement at the time of the experiment was "safe."

*Experiment 7.*—This air sample was taken under practically the same conditions as in the previous experiment except that only about 50 people were in the crowd. The plate examined contained 2 streptothrix, 13 bacillus, and 8 coccus colonies. All of the cocci gave negative reactions throughout on the three differential media. No salivary coccus forms were found, which fact leads again to the conclusion that the sanitary quality of the air during the experiment was "safe."

EXPERIMENTS 2, 12, 13, 14

These experiments were performed outdoors. The air sample for experiment 2 was collected in a railroad switch yard at a time when there was no traffic. The samples for experiments 12, 13, and 14 were collected in the immediate vicinity of large storage basins belonging to the local water works and located 300 or 400 feet from the bank of the Mississippi River. The apparatus used was the same as that employed in the previous experiments.

*Experiment 2.*—The outdoor temperature was 29°F., and while the air sample was being taken it was snowing. A total of 22,500 cc. of air was drawn through the sand filter at the rate of 750 cc. per minute—the operation extending over a period of 30 minutes. Samples were plated as shown in table IX. Of the 3 plates examined, plate 1 yielded 2 bacillus colonies; plate 2, 1 streptothrix, 1 bacillus, and 6 coccus colonies; and plate 4, 2 mold, 1 streptothrix, 1 bacillus, and 2 coccus colonies. All of the coccus forms were grown on the three differential media, 7 giving negative reactions throughout, while 1 reacted positively on saccharose and negatively on lactose and milk. The latter organism is one of the unidentified coccus forms previously referred to. No characteristic salivary cocci were found, in-

dicating that the sanitary quality of the air examined was "safe."

*Experiment 12.*—At the time the air sample was being taken, a slight drizzling rain was falling, accompanied by considerable wind and a temperature of 45°F. Prior to that time it had been raining continuously for about 24 hours. A total of 10,800 cc. of air was drawn through the sand filter at the rate of 900 cc. per minute, the apparatus meanwhile being held about 5 feet above the ground level. The sand of the filter was introduced into 15 cc. of sterile distilled water, from which platings were made. Table VIII gives the details of the experiment, together with the results obtained.

TABLE VIII

| Plate number | Quantity plated | Total no. of colonies | No. of bacteria and molds | Coccus colonies | No. of salivary cocci |
|---|---|---|---|---|---|
| 1 | 1 cc. | 9 | 2 | 7 | 7 |
| 2 | 1 cc. | 3 | 3 | 0 | 0 |
| 3 | 5 cc. | 0 | 0 | 0 | 0 |
| 4 | 5 cc. | 6 | 4 | 2 | 0 |

Attention should be called to the fact that on plate 1, in which only 1 cc. of the solution was used, 9 colonies developed—7 coccus and 2 bacillus—, while on plate 4, in which 5 cc. of the solution were used, only 6 colonies appeared,—2 coccus and 4 bacillus. Furthermore, the 7 colonies in plate 1 proved to be of salivary cocci, whereas none of these organisms were present among the cocci of plate 5. These results unquestionably indicatelocal contamination. It is difficult to say just where the contamination took place. Obviously it did not occur during the collection of the sample or even during the mixing of the sand solution; for had this been the case all of the plates should have shown salivary cocci, and the greater number should have occurred on those plates in which larger quantities of the solution were plated. In all probability plate 1 was locally contaminated.

*Experiment 13.*—While the sample of air was being taken for this experiment, the temperature was 63°F., a light breeze was blowing, and the sky was very cloudy although no rain had

fallen during the preceding 18 hours. A total of 10,800 cc. of air was drawn through the apparatus at the rate of 830 cc. per minute. Samples of the sand solution were plated as shown in table IX. It is to be noted that in those plates containing 1 cc. of the solution no colonies developed, whereas in those containing 5 cc., 1 bacterial colony appeared in each. Attention is called to the consistent results in this experiment to emphasize the fact that the inconsistencies in experiment 12 are due to local contamination. No salivary cocci were found.

*Experiment 14.*—The air sample for this experiment was taken on a bright, clear day, with a rather strong wind blowing and a temperature of 55°F. A total of 10,800 cc. of air was drawn through the sand filter at the rate of 1,080 cc. per minute. Samples of the sand solution were plated as shown in table IX. Of the 3 coccus forms, 2 gave negative reactions on all three differential media, whereas 1 was positive on saccharose and negative on lactose and milk. The latter will be recognized as one of the unidentified coccus forms. No salivary cocci were isolated.

Summarizing the open air experiments, it is to be noted that, barring the locally contaminated plate 1 in experiment 12, the characteristic salivary coccus form was not isolated; furthermore, that the total number of organisms in the open air is comparatively low.

## Summary and Conclusions

Examining the entire series of experiments it appears that in the majority of cases where ventilation was obviously inadequate, the characterisitic salivary coccus form was isolated. On the other hand, the form could in no case be found where ample artificial or natural ventilation existed.

It has been shown that the most characteristic salivary organism can be differentiated and identified; also, that this characteristic organism can be isolated from the air.

In the experiment carried on in one of the street cars in which there were many passengers, the characteristic salivary coccus form was found to be present in such quantities as to indicate that the air in this car was "unsafe." It was later shown that

## TABLE IX

DATA ON THE COLLECTION AND EXAMINATION OF AIR SAMPLES

| No. of experiment | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| Date of collection | | 2/12/13 | 2/22/13 | 3/15/13 | 3/15/13 | 3/19/13 | 3/22/13 | 3/22/13 |
| Sampling place | | Lab. | Outdoors | Street car | 5 and 10c. store | Street car | Street car | 5 and 10c store |
| Temperature (°F.) | Outdoors | 60 | 29 | 32 | 36 | 50 | 23 | 50 |
| | Sampling pl. | 80 | 29 | 45 | 70 | 48 | 45 | 70 |
| Weather conditions | | Sunshine | Snowing | Windy snowing | Windy snowing | Sunshine | Sunshine | Sunshine |
| Approx. no. of persons | Sitting | 0 | 0 | 44 | 1 | 44 | 44 | 1 |
| | Standing | 2 | 0 | 0 | 100 | 30 | 30 | 50 |
| Approx. volume of sampling pl. (cu. ft.) | | 3000 | | 2500 | 72000 | 2500 | 2500 | 72000 |
| Volume of air exam. (cc.) | | 7800 | 22500 | 10800 | 10800 | 10800 | 10800 | 10800 |
| Rate of filtration (cc. per min.) | | 520 | 750 | 900 | 900 | 900 | 900 | 900 |
| No. of organisms in following quantities of sand solution plated | Pl. I. (1 cc.) | 2 | 2 | 4 | 30 | 9 | 250 | 4 |
| | Pl. II. (1 cc.) | 2 | 8 | 3 | 36 | 6 | 250 | 3 |
| | Pl. III. (2 cc.) | 4 | 0 | | | | | |
| | Pl. IV (5 cc.) | | 6 | 12 | 230 | 25 | Too numerous to count. | Spreader |
| | Pl. V. (5 cc.) | | | 20 | 200 | 26 | Too numerous to count. | 23 |
| Plates examined | | I., II. and III. | I., II. and IV. | V. | II. | V. | 1/12 of I. | V. |
| Total col. on plates exam. | | 8 | 16 | 20 | 36 | 26 | 21 on 1/12 of I. | 23 |
| No. of bacilli, molds, etc. | | 4 | 8 | 9 | 16 | 16 | 5 on 1/12 of I. | 15 |
| No. of cocci | | 4 | 8 | 11 | 20 | 10 | 16 on 1/12 of I. | 8 |
| No. of salivary cocci | | 1 | 0 | 2 | 0 | 0 | 6 on 1/12 of I. | 0 |
| Frequency of occurrence | | 1 in 1950 | 0 | 1 in 1800 | 0 | 0 | 1 in 10 | 0 |
| Sanitary quality | | Probably unsafe | Safe | Probably unsafe | Safe | Safe | Unsafe | Safe |
| No. of org. in total vol. of air | | 30 | 42 | 50 | 570 | 95 | 3750 | 58 |

TABLE IX (*Continued*)

DATA ON THE COLLECTION AND EXAMINATION OF AIR SAMPLES

| No. of experiment | | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|
| Date of collection | | 3/29/13 | 3/29/13 | 4/1/13 | 4/1/13 | 4/8/13 | 4/9/13 | 4/10/13 |
| Sampling place | | Street car | Picture show | Street car | Picture show | Outdoors | Outdoors | Outdoors |
| Temperature (°F.) | Outdoors | 41 | 59 | 50 | 77 | 45 | 63 | 55 |
| | Sampling pl. | 55 | 70 | 63 | 82 | 45 | 63 | 55 |
| Weather conditions | | Sunshine | Cloudy | Sunshine | Sunshine | Rain, very windy | Cloudy | Sunshine, very windy |
| Approx. no. of persons | Sitting | 44 | 3000 | 44 | 3000 | 0 | 0 | 0 |
| | Standing | 30 | 100 | 15 | 300 | 0 | 0 | 0 |
| Approx. volume of sampling pl. (cu. ft.) | | 2530 | 90000 | 2500 | 90000 | | | |
| Volume of air exam. (cc.) | | 10800 | 10800 | 10800 | 10800 | 10800 | 10800 | 10800 |
| Rate of filtration (cc. per min.) | | 900 | 900 | 900 | 900 | 900 | 830 | 1080 |
| No. of organisms in following quantities of sand solution plated | Pl. I. (1 cc.) | 34 | 3 | 4 | 7 | 9 | 0 | 2 |
| | Pl. II. (1 cc.) | 18 | 2 | 2 | 3 | 3 | 0 | 2 |
| | Pl. III. (2 cc.) | 24 | | | | | | |
| | Pl. IV. (5 cc.) | Spreading mold | 14 | Spreader | Spreader | 0 | 1 | 3 |
| | Pl. V. (5 cc.) | | 8 | 12 | 35* | 6 | 1 | 1 |
| Plates examined | | I. | IV. | V. | I. and II. | I., II. and V. | IV. and V. | I.,II.,IV., and V. |
| Total col. on plates exam. | | 34 | 14 | 12 | 10 | 18 | 2 | 8 |
| No. of bacilli, molds, etc. | | 15 | 12 | 5 | 9 | 9 | 2 | 5 |
| No. of cocci | | 19 | 2 | 7 | 1 | 9 | 0 | 3 |
| No. of salivary cocci | | 0 | 0 | 1 | 0 | 7 | 0 | 0 |
| Frequency of occurrence | | 0 | 0 | 1 in 3600 | 0 | 1 in 1235 | 0 | 0 |
| Sanitary quality | | Safe | Safe | Question-able | Safe | † | Safe | Safe |
| No. of org. in total vol. of air | | 320 | 35 | 42 | 85 | 50 | 2 | 18 |

* Abundance of molds.
† Local contamination.

the salivary coccus form could not be found in the open air devoid of the immediate presence of human beings.

It thus appears that the presence of the salivary coccus form in air indicates the presence of man; furthermore, it indicates the pollution of air by particles of mucus from the mouth.

Flügge [1] and his school have shown that pathogenic organisms may be transmitted into the air, and other workers [2] have shown that the tubercle organism is capable of being carried by even such feeble air currents as ordinarily exist in dwellings.

The tubercle organism, as well as the characteristic salivary organism, is present in the saliva of tubercular patients. If, therefore, this salivary organism can be isolated from the air by means of the filter used in the above experiments, does it not follow that the tubercle organism could be isolated in a similar way? Since our manner of breathing is comparable to the operation of the apparatus used, it follows that the tubercle organism may be inhaled by man.

It thus appears that the presence in the air of the most characteristic salivary organism is an index of the possible access of pathogenic organisms to the atmosphere.

In conclusion, the writer wishes to express his thanks to Dr. Geo. T. Moore, for valuable suggestions and numerous courtesies extended during the progress of the work; to Dr. J. R. Schramm, for suggestions, and aid in the preparation of the manuscript; and to Mr. Wilson F. Monfort, Chemist of the City of St. Louis Water Department, for advice given and opportunities provided for the collection and examination of the samples.

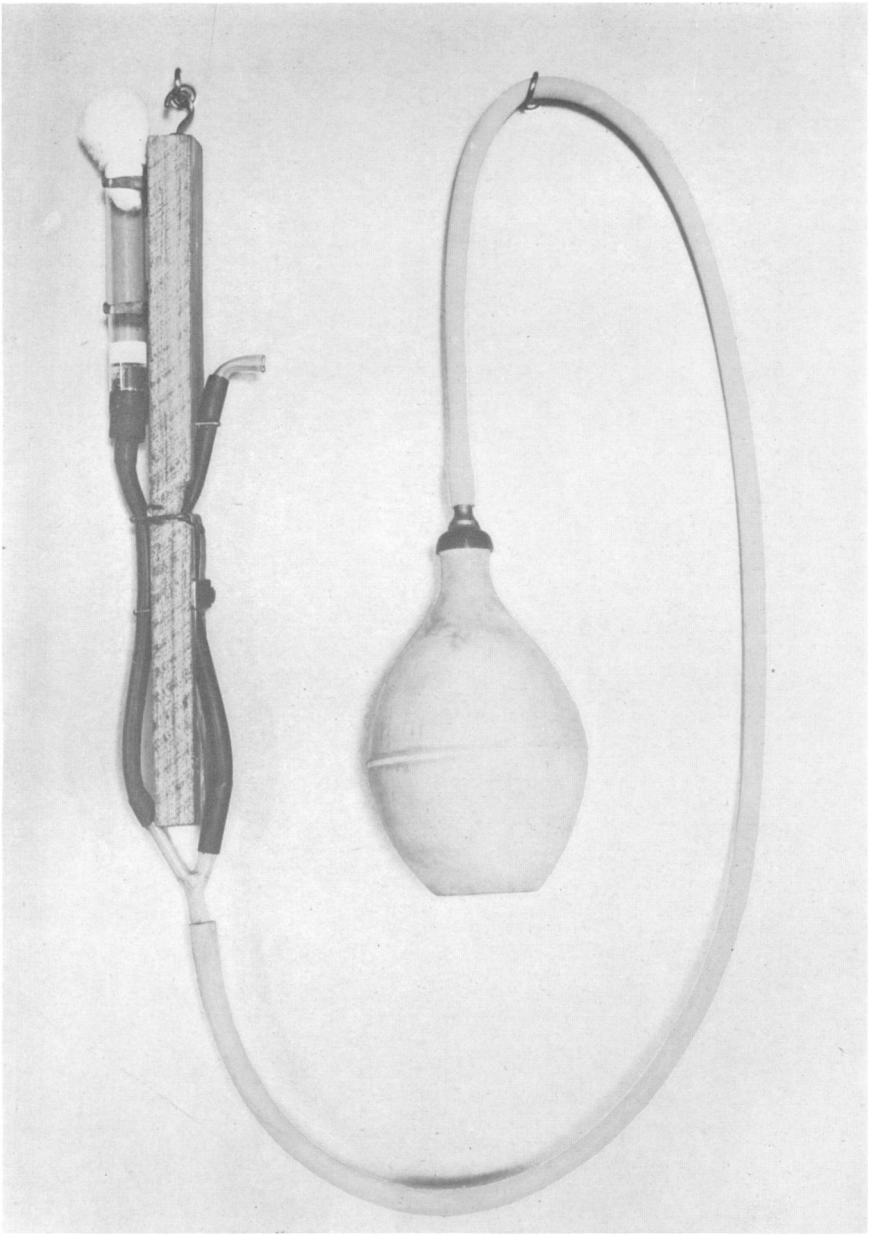
## EXPLANATION OF PLATE

### PLATE 2

Air-sampling apparatus showing support, sand filter, pinch cock, exhaust and suction tubes, and pressure bulb.

[1] Gordon, M. H. *loc. cit.*

[2] Kolle and Wassermann, Handbuch der pathogenen Mikroorganismen 1: 169.



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